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On JANUARY 10, 2001

TOWNSEND and TOWNSEND and CREW LLP

By: Karen Dorino

PATENT
Attorney Docket No.: 012176-006310
Client Ref. No.: AO156-1

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

*In re* application of:

Gutterson *et al.*

Application No.: 09/012,895

Filed: January 23, 1998

For: TWO COMPONENT PLANT CELL LETHALITY METHODS AND COMPOSITIONS

Examiner: Zaghmout, O.

Art Unit: 1649

DECLARATION OF DR. NEAL GUTTERSON UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

I, Neal Gutterson, Ph.D., being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I, Dr. Gutterson, am currently Director of Research at DNA Plant Technology Corporation. I have been in this position for approximately one and a half years and have been employed by DNA Plant Technology Corporation for over 17 years. In 1982,

I graduated from the University of California at Berkeley with a Ph.D. degree in Biochemistry. A copy of my curriculum vitae is attached hereto as Exhibit 1.

3. The present invention provides for the first time a two polypeptide component system to produce a desired phenotype in plant cells, e.g., a lethal effect. In this system, two different polypeptides are expressed in a plant cell. Each expression cassette of the invention is individually functional, but the product of each cassette alone does not provide the desired effect or change the phenotype of the cell. The combination of the two polypeptides from the individual expression cassettes is required for producing the desired phenotype. The first and second polypeptides can either be separate functional polypeptides, or nonfunctional polypeptide subsequences that together produce a single functional polypeptide.

4. I am a named inventor on the above-referenced patent application. I have read and am familiar with the contents of this patent application. In addition, I have read the Office Action, dated June 20, 2000, received in the present case. It is my understanding that the Examiner is concerned that the claimed methods are not enabled by the specification.

5. This declaration is provided to demonstrate that practice of the claimed methods is fully enabled by the specification. This declaration presents plant experiments in which two separate subsequences of a single functional protein (barnase, a ribonuclease) were expressed in transgenic plants. The experiments described herein were done under my supervision. In one experiment, the barnase polypeptides were expressed in transgenic tomato plants using a constitutive promoter. In another experiment, the barnase polypeptides were expressed in transgenic tomato plants using a tapetal-specific promoter. In both experiments, the polypeptide subsequences formed a functional protein that impaired cellular function in the cells in which the polypeptide subsequences were expressed. One of skill in the art can therefore practice the claimed methods using information provided in the specification,

together with methodology known to one of skill in the art, with, at most, only routine experimentation. The claimed methods are thus fully enabled by the specification.

6. In one experiment, expression of 5' and 3' barnase polypeptide subsequences in the same cells, using a constitutive promoter, was lethal to the plant. Expression vectors were made that encoded either a 5' barnase polypeptide (p35S/Bn5-2) or a 3' barnase polypeptide (p35S/Bn3-2), each operably linked to the Cauliflower mosaic virus 35S promoter (a constitutive promoter). Transgenic tomato plants were transformed with either the 5' barnase expression vector or the 3' barnase expression vector, according to standard techniques, and these plants expressed their respective integrated transgenic constructs.

7. To determine whether expression of Bn5-2 and Bn3-2 together using a constitutive promoter would be lethal to the plant, transgenic tomato plants expressing either p35S/Bn5-2 or p35S/Bn3-2 were crossed, and the resulting seeds were planted. In one cross, thirty nine seeds were planted. Both parents were hemizygous and had transgenes at two loci, so that approximately 55% of the progeny were expected to inherit both transgenes. Twenty (51%) of the seeds did not germinate. Among the 19 seeds that did germinate, none carried both transgenes. For all crosses combined, out of 87 seeds planted, only 35 of these seeds germinated and produced normal-looking tomato plants (*see* Table 1, Exhibit 2). None of these normal plants carried both the Bn5-2 and the Bn3-2 constructs. Besides the normal looking tomato plants, two seeds germinated but died after making only a hypocotyl and a few roots. PCR analysis detected both the Bn3-2 and the Bn5-2 constructs in those two plants. These results are consistent with the prediction that the combination of both transgenes would be lethal to the plant.

8. In another experiment, expression of the 5' and 3' barnase polypeptide subsequences together in specific cells (the tapetum), using a tissue specific promoter, resulted in impairment of the cells and male sterility. As described above, transgenic tomato plants

were transformed according to standard methodology with either a Bn5-2 or a Bn3-2 transgene construct operably linked to a p127a promoter, a tomato tapetal-specific promoter. Expression of both the Bn3-2 and the Bn5-2 integrated transgenes peaked at the 5-7 mm bud stage, which corresponds to the tetrad stage of microspore development when the tapetum is maximally active.

9. To determine whether expression of Bn5-2 and Bn3-2 together in the tapetum would be lethal to pollen generation, transgenic tomato plants expressing either p127a /Bn5-2 or p127a /Bn3-2 were crossed, and the resulting seeds were planted. In one cross, one of three seeds germinated. This plant inherited both Bn5-2 and Bn3-2 and was male sterile. The anthers on the plant were flat but not reduced in size. The plant had normal flower and plant morphology. It did not produce any selfed fruit, but was female fertile, producing fruit when outcrossed as a female. Seed has been successfully germinated from the outcrossed fruit. Additional experiments have demonstrated co-segregation of: (1) the two transgenes (Bn5-2 and Bn 3-2) and (2) the male sterile phenotype.

10. When a flower from the Bn5-2/Bn3-2 plant was examined, only 64 pollen-like grains were found. These grains differed morphologically from normal pollen grains, being less refractile, smaller, and shriveled in appearance. None of these pollen grains were viable, according to a fluorescein diacetate staining assay. In comparison, control flowers, including controls corresponding to both parents, had between 2400 to 7300 pollen grains per flower, with 39-51% of the pollen grains being viable according to the fluorescein diacetate staining assay. These results are consistent with the prediction that the combination of both transgenes would be lethal to pollen generation.

11. In view of the foregoing, it is my scientific opinion that one of skill in the art would be able to practice the claimed invention with, at most, routine experimentation. The specification therefore fully enables the methods of the invention.

Date: January 5, 2001

By: _____
Neal Gutterson, Ph.D.

SF 1169756 v1

# CURRICULUM VITAE
## Neal Gutterson

### Education

| | |
|---|---|
| University of California, Berkeley | Ph.D., Biochemistry, 1982 |
| Yale University | B.S., Chemistry, 1977 |

### Professional Experience

| | |
|---|---|
| 1999- present | Director of Research<br>DNA Plant Technology Corp., Oakland, CA |
| 1992 - 99 | Managing Research Director<br>Vegetatively Propagated Plants/New Crops<br>DNA Plant Technology Corp., Oakland, CA |
| 1990 - 92 | Senior Research Director<br>Biochemical Genetics (and Floriculture)<br>DNA Plant Technology Corp., Oakland, CA |
| 1988 - 90 | Principal Scientist, Microbial Genetics<br>DNA Plant Technology Corp., Oakland, CA |
| 1984 - 88 | Research Scientist, Microbial Genetics<br>DNA Plant Technology Corp., Oakland, CA<br>(formerly Advanced Genetic Sciences) |
| 1983 - 84 | Postdoctoral Fellow<br>Advanced Genetic Sciences, Oakland, CA |

### Memberships

International Society of Plant Molecular Biology
Sigma Xi

### Patents

Tucker, W. and N. Gutterson. U.S. patent 5,102,797. Introduction of heterologous genes into bacteria using transposon flanked expression cassette and a binary vector. Issued April 7, 1992.

Gutterson, N., Tucker, W., and P. Wolber. U.S. patent 5,187,061. Transducing particles and methods for their production. Issued February 16, 1993.

Chuck, G., Dooner, H., Gutterson, N., Keller, J., Nijjar, C., Ralston, E. U.S. patent 5,534,660. pH genes and their uses. Issued July 9, 1996.

Michael, M.Z., M.W. Graham, E.C. Cornish, N.I. Gutterson, W.T. Tucker. EP0824591. Transgenic Carnations exhibit prolonged post-harvest life.

Chuck, G., Dooner, H., Gutterson, N., Keller, J., Nijjar, C., Ralston, E. U.S. patent 5,910,627. pH genes and their uses. Issued June 8, 1999.

Firoozabady, E, Gutterson, N. Genetically transformed pineapple plants and methods for their production. U.S. patent 5,952,543. Issued Sept. 14, 1999.

**Patent applications filed**

Burgess, D., Gutterson, N., and Ralston, E. Materials and methods for hybrid seed production. WO9832325. Priority date, November 14, 1997.

Engler, D., Gutterson, N., Nisbet, G. Method of genetically transforming banana plants. WO9903327. Priority Date, July 16, 1997.

Gutterson, N. and Ralston, E. Two component plant cell lethality methods and compositions. Priority date, January 24, 1997.

**Recent Publications**

Hanson, B., Engler, D., Moy, Y., Newman, B., Ralston, E. and Gutterson, N. 1999. A simple method to enrich an *Agrobacterium*-transformed population for plants containing only T-DNA sequences. The Plant Journal, accepted for publication.

Firoozabady, E, Moy, Y., Tucker, W., Robinson, K., and Gutterson, N. 1995. Efficient transformation and regeneration of carnation cultivars using *Agrobacterium*. Molecular Breeding, 1, 283-93.

Gutterson, N. 1995. Anthocyanin biosynthetic genes and their application to flower color modification through sense suppression. HortScience, 30, 964-66.

Robbins, T.P., Jenkin, M., and Courtney-Gutterson, N. 1994. Enhanced frequency of transposition of the maize transposable element *Activator* following excision. Mol. and Gen. Genet., 244, 491-500.

Firoozabady, E., Moy, Y., Courtney-Gutterson, N., and Robinson, K. 1994. Regeneration of transgenic rose (Rosa hybrida) plants from embryogenic tissue. Bio/Technology, 12, 609-13.

Courtney-Gutterson, N., Napoli, C., Lemieux, C., Morgan, A., Firoozabady, E., and Robinson, K. 1994. Modification of flower color in Florist's Chrysanthe-mum: production of a white-flowering variety through molecular genetics. Bio/Technology, 12, 268-71.

Courtney-Gutterson, N. 1994. The biologists's palette: genetic engineering of anthocyanin biosynthesis and flower color, in *Genetic Engineering of Plant*

*Secondary Metabolism*, Ellis, B.E., Kuroki, G.W., and Stafford, H.A., eds. Plenum Press, New York, pp. 93 - 124.

Chuck, G., Robbins, T., Nijjar, C., Ralston, E., Courtney-Gutterson, N., and Dooner, H.K. 1993. Tagging and cloning of a petunia flower color gene with the maize trasposable element *Activator*. The Plant Cell, 5, 371-78.

Courtney-Gutterson, N. 1993. Molecular breeding for color, flavor and fragrance. *Scientia horticulturae*, 55, 141-60.

Table I. *35S-Bn* Crosses

| Cross | # Seeds | # Germinated (normal) | # Inserts or Loci | | Number with: | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Parent 1 | Parent 2 | Bn5-2 only | Bn3-2 only | Neither | Both |
| 41-6 x 45-4 | 2 | 2 | 1 locus | 1 locus | 2 | 0 | 0 | 0 |
| 41-9 x 45-4 | 11 | 7 | 1 locus | 1 locus | 3 | 3 | 1 | 0 |
| 42-6.2 x 40-3.1 | 5 | 3 | 1 locus | 1 locus | 0 | 3** | 0 | 0 |
| 42-12 x 40-2 | 4 | 1 | 1 locus | 1 locus | 0 | 1 | 0 | 0 |
| 45-26.3 x 40-3.6 | 12 | 3* | 1 locus | 1 locus | 0 | 3** | 0 | 0 |
| 71-11.6 x 40-7.6 | 39 | 19 | 2 loci | 2 loci | 8 | 6 | 5 | 0 |
| 71-17 x 40-4 | 10 | 0 | 5 inserts | 1 locus | | | | |
| 71-16 x 40-7 | 1 | 0 | 4 inserts | 2 loci | | | | |
| 71-24 x 40-7 | 1 | 0 | 5 inserts | 2 loci | | | | |
| 71-19 x 40-7 | 2 | 0 | 2 inserts | 2 loci | | | | |
| Total | 87 | 35 | | | 13 | 16 | 6 | 0 |

40: p35S/Bn3-2/ocs3'
41: d35S/int/Bn3-2/nos3'

45: p35S/Bn5-2/ocs3'
42: d35S/int/Bn5-2/nos3'
71: p35S/Bn5-2/GpII3'

* 2 others died after producing a hypocotyl and roots; both had Bn5-2 plus Bn3-2

** possibly homozygous for Bn3-2